Amendments to the Specification

Please add the following new paragraphs at page 1 line 2 of the application (immediately after the Title):

Cross-Reference to Related Applications

This application is a national stage entry of PCT Application No. PCT/EP00/00598, filed January 26, 2000, which claims priority from European Patent Application No. EP 99101480.4, filed January 27, 1999.

Please replace the paragraph occurring at page 1 lines 12-17 with the following amended paragraph:

Several documents are cited throughout the text of this specification. Each of the documents cited herein (including any manufacturer's specifications, instructions, etc.) are hereby incorporated by reference; however, there is no admission that any document cited is indeed prior art of the present invention. Further incorporated by reference is the complete disclosure content of the co-pending application filed in the name of IDEA AG and bearing the title "Noninvasive vaccination through skin" (U.S. Appln. No. 09/890,335; published as WO 00/44349).

Please replace the paragraph occurring at page 13 lines 21-26 with the following amended paragraph:

Formulations including the above-referenced penetrants are described in detail in DE 41 07 152, PCT/EP91/01596 (published as WO/1992/003122 and equivalent to U.S. Patent No. 6,165,500 A), PCT/EP96/04526 (published as WO/1998/17255 and equivalent to U.S. Publication No. 2002/048596), and DE 44 47 287, which are ~~incorproated~~ incorporated herewith by reference. Relevant information useful for penetrant manufacturing and loading with various macromolecular actives, which are too big to permeate through the barrier, is given in patent application PCT/EP98/06750 (published as WO 00/24377 and equivalent to U.S. Publication No. 2008/279815), also incorporated herewith by reference.

Please replace the paragraph occurring at page 38 lines 7-10 with the following amended paragraph:

The disclosure content of the documents cited throughout this specification are herewith incorporated by reference. Further incorporated by reference is the complete disclosure content of the co-pending application filed in the name of IDEA AG and bearing the title "Noninvasive vaccination through the skin" (U.S. Appln. No. 09/890,335; published as WO 00/44349).

Please replace the paragraph occurring at page 38 lines 23-25 with the following amended paragraph:

Figures 3a and 3b provide further examples measured with a healthy volunteer (Figure 3a) and an insulin-dependent diabetes mellitus patient (Figure 3b) following intranasal administration of insulin formulations with inferior characteristics, believed to be due to too slow drug release from the carrier.

Please replace the paragraph occurring at page 39 lines 1-4 with the following amended paragraph:

Figures [[~~7~~]] 7a, 7b and 7c illustrate the effect of changing aggregate size and/or deformability on TT specific immune response in mice treated with various mixed micelles, Transfersomes or liposomes loaded with TT. Panels a and b show antibody isotype patterns, and in panel c the total antibody titre, as expressed in absorbency change is given.

Please replace the paragraph occurring at page 39 lines 6-10 with the following amended paragraph:

Figures [[~~8~~]] 8a, 8b and 8c highlight the (small) effect of changing antigen dose (in the high dosage range) on transnasal immunization of mice with TT by means of Transfersomes with or without lipid A derivative as an immunoadjuvant. In panel a, the results of total absorbance measurements are given, panel b shows the corresponding titration curves, and panel c gives the relevant antibody isotypes.

Please replace the paragraph occurring at page 39 lines 12-13 with the following amended paragraph:

Figures 9a, 9b and 9c are ~~Figure 9 is~~ organized in similar fashion to compare the outcome of intranasal, oral or subcutaneous TT administration using different antigen doses and purity.

Please replace the paragraph occurring at page 39 lines 15-16 with the following amended paragraph:

Figures 10a and 10b: ~~Figure 10:~~ For comparison, animal protection (survival) data are given for the experiments in which several doses and administration routes were compared.

Please replace the paragraph occurring at page 39 lines 18-21 with the following amended paragraph:

Figures 11a and 11b present ~~Figure 11 presents~~ a set of data on the effect of various cytokines, or their combination, on the murine immune response to TT administered into the nose by means of transfersomes, with s.c. data given for comparison. Panel a gives the absorbance and titre data and panel b contains the isotype distribution results.

Please replace the paragraph occurring at page 41 lines 27-33 with the following amended paragraph:

To follow the temporal variation of glucose concentration in the blood, 5 µL to 30 µL [[µ]]samples taken, every 10 min to 15 min, from the fingers on both arms. After an initial test period, during which the 'normal' blood glucose concentration and/or its change was determined, a suspension of carriers loaded with insulin (Transfersulin) was sprayed into each nostril, using conventional non-metered sprayer, in a series of 150 µL puffs. Care was taken to minimise the spill-over of test formulation into the throat or the dropping of said formulation from the nose.

Please replace the paragraph occurring at page 42 lines 6-11 with the following amended paragraph:

The test formulations were made essentially as described in patent application PCT/EP98/06750 (published as WO 00/24377 and equivalent to U.S. Publication No. 2008/279815). In brief, a suspension of highly adaptable penetrants with the above mentioned composition and an average diameter of the order of 100 nm to 150 nm was loaded with the drug, based on interfacial adsorption, and used within 24 h after the preparation. The drug-carrier association in the formulation was determined to be between 60% and 70%.

Please replace the paragraph occurring at page 42 line 33 – page 43 line 7 with the following amended paragraph:

Results measured with a healthy subject are shown in ~~figure 1~~ Figure 2. They reveal a transient decrease in the systemic blood glucose concentration after two administrations of the drug in carriers (closed symbols), with a maximum after 20-30 min and a return to the pre-treatment value after approximately 1 h in either case. The observed change in glucose level corresponds to approximately 8.5% of the decrease was measured in an independent experiment after intravenous injection of the drug (inset: open symbols). The reproducibility remains to be improved, however, the first application, biased by the lack in administration skill having been less successful than the second administration.

Please replace the paragraph occurring at page 43 line 27 – page 44 line 4 with the following amended paragraph:

Results of an experiment done with said IDDM patient is illustrated in ~~figure 2~~ Figure 1. Owing to the lack of endogenic insulin production in this test subject, the pre-treatment blood glucose concentration was slightly above the normal, but relatively constant. The change resulting from nasal drug administration with ultra-adaptable carriers, has more a step-like rather than a peak-like shape (closed symbols), completed within 75 min. This is precisely what one would expect for an IDDM patient. The result of an i.v. injection of rapidly acting insulin (Actrapid™, Novo-Nordisk) in the same test person on a different occasion (inset: open symbols) corroborates the conclusion. An estimate of apparent bioavailability of nasal insulin based on these data is around 4% and, consequently, somewhat lower than that reported in example 1. This may have to do with the presumed variability in drug release between different formulations which is illustrated in the following examples.

Please replace the paragraph occurring at page 44 lines 20-28 with the following amended paragraph:

Results of the test measurements done with several different vesicle suspensions, illustrated in ~~figure 3~~ Figures 3a and 3b, signal lack of action for the insulin administered nasally with such carriers. The blood glucose concentration in the investigated normoglycaemic test person remains the same before, during and after the drug administration, for several hours at least. This suggests that the mere presence of carriers, or their ingredients, is insufficient to improve the bioavailability of nasally applied macromolecules, such as insulin. To achieve the desired biological effect, the rate of drug release from

the carrier must also be adequate, such rate being determined in in dedicated ex vivo experiments by using conventional protein binding deassociation techniques.

Please replace the paragraph occurring at page 45 line 32 - page 46 line 7 with the following amended paragraph:

Results pertaining to different time-points are given in ~~Figure 4~~ Figure 5. They show that substantial amount of nasally administered radioactivity is recovered from the body, even after exclusion of gastro-intestinal tract, especially during the first hours following suspension administration. Values in the blood are in the range of 9% at 0.5 h and 2%, the specific concentration falling from 3%/mL at the beginning to 0.7%/mL at the end. Activity in the nose decreases from 10.4% at 0.5 h to 0.3% at 8 h. Liver values are between 2.3% after 0.5 h, the maximum around 2.8 at 1 h and values above 1% after 4 h. After 8 h, the residuum in the liver is around 0.4%. The relatively high hepatic values are suggestive of passage of particles, that is, penetrants, through the barrier and subsequent uptake in the reticulo-endothelial system.

Please replace the paragraph occurring at page 47 lines 23-26 with the following amended paragraph:

Measured radioactivity in the blood was found to correspond to app. 2.5% of the applied dose, liver concentration being at app. 2% and colon concentration around 2.5%, all after 2 h. The highest amount of radioactivity by then was recovered from the stomach (37%) and in the cage plus excrement (32%). See Figure 6.

Please replace the paragraph occurring at page 48 lines 1-9 with the following amended paragraph:

highly adaptable penetrants

- 37.7 mg/mL phosphatidylcholine from soy bean (SPC)
- 62.3 mg/mL polysorbate (Tween 80)
- phosphate buffer, 10 mM, pH 6.5
- Tetanus toxoid, as antigen (2 mg/mL)
- Interferon-γ ~~(IFG-γ)~~ (IFN-γ)
- Granulocyte-monocyte-colony stimulating factor (GM-CSF)
- Interleukin 4 (IL-4)
- Interleukin 12 (IL-12)

Please replace the paragraph occurring at page 48 lines 22-29 with the following amended paragraph:

The results of above mentioned measurements, illustrated in ~~figure 6~~ Figure 4, suggest that the presence of all tested cytokines in vaccination formulation, based on the highly adaptable antigen carriers, increases the serum absorbance compared to that characterizing the non-modulated value, determined after simple immuno-carrier administration. Relative differences are more likely

consequences of diverse bio-potency of tested immuno-modulants employed in the present specific experimental system than indicative of variable macromolecular transport rate across the nasal mucosa.

Please replace the paragraph occurring at page 51 lines 26-33 with the following amended paragraph:

Mixed micelles containing less potent detergents (with lesser skin permeation enhancing capability) are, relatively speaking, less efficient 'immuno-carriers' (see figure 7a [[~~7b~~]]), the more deformable Transfersomes with a higher Tw content standing clearly out in the case of IgG2a and IgM, are similar to the less deformable Transfersomes with a lower Tw content in the case IgG1 and IgG3, and are as efficient as mixed micelles with Tw in the case of IgA and IgG2b. The smallness of measured values is reason for the concern, however, which can best be overcome by using purer antigen.

Please replace the paragraph occurring at page 53 lines 23-30 with the following amended paragraph:

The results are given in figures 9 a – c. They reveal that the increase in serum absorbance, ultimately, is comparable after invasive and non-invasive antigen administration (figure 9a). However, the titre in the former ~~latter~~ case is significantly lower after the first boost ~~except in the primary response~~. Interestingly, s.c. injection only produces superior results after the second boost, whereas the combination with TT and cholate, which then can act as nasal permeation enhancer in total antibody titre is better at all times. The probable reason for this is the high concentraton of IgG2b, as is seen from figure ~~9b~~ 9c. Injections elicit most efficiently the IgG1 and IgM type of antibodies.

Please replace the paragraph occurring at page 53 line 32 – page 54 line 2 with the following amended paragraph:

Animals are well protected by any of above mentioned vaccinations with TT, but only after 2 boosts; in the case of nasal vaccination. In contrast, one boost is sufficient (data not shown). Using 4-8x lower doses of purified TT suffices for protection in the case of nasal vaccination, but not when the antigen is injected (cf. Figure 10a [[~~10~~]].

Please replace the paragraph occurring at page 55 lines 1-8 with the following amended paragraph:

The effect of cytokines was studied individually and in combination. The results are given in figures [[~~5~~]] 11a and 11b. They suggest that GM-CSF plus IL-4 combination can support the generation of anti-TT antibodies in mice, as can, probably, IFN-γ and perhaps IL-12, and maybe IL-4 (cf. figure 11a). The strongest effect is seen in the case of IgM and IgA, except in the case of IL-12 usage, which only affects strongly IgG2b generation. The protection relevant IgG1 is increased strongly only by the combination of GM-CSF and IL-4, whereas IgG3 is not affected at all. ~~Injection~~ Intranasal administration works best for IgG1 (cf. figure 11b).

Please replace the paragraph occurring at page 55 lines 22-26 with the following amended paragraph:

The effect discussed with examples 30-35 ~~25-31~~ was confirmed for a blend low molecular and high molecular weight immunoadjuvants. The results are given in figures 12 and show that the immunopotentiation by such a combination is especially strong during the early stage of immune response, the combination in any case being better than LA alone.

Please replace the paragraph occurring at page 56 lines 21-27 with the following amended paragraph:

The protective effect of antigen applied in the nose was good when the antigen dose exceeded 20 μg/immunisation; lower doses yielded insufficient, but detectable protection (cf. figure 13). When cholera toxin (CT) was included into the test formulation together with the tetanus toxoid, excellent protection was achieved already at the lowest of tested doses (0.5 μg/immunisation), independent of the ultra-deformable carrier composition. Protection was complete in all test groups containing CT in the formulation applied subcutaneously ~~on the skin~~.